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# 1 *Daphnia* invest in sexual reproduction when its relative costs are reduced

2

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11

## 12 **Abstract**

13 The timing of sex in facultatively sexual organisms is critical to fitness, due to the differing  
14 demographic consequences of sexual vs. asexual reproduction. In addition to the costs of sex itself,  
15 an association of sex with the production of dormant life stages also influences the optimal use of  
16 sex, especially in environments where resting eggs are essential to survive unfavourable conditions.  
17 Here we document population dynamics and the occurrence of sexual reproduction in natural  
18 populations of *Daphnia magna* across their growing season. The frequency of sexually reproducing  
19 females and males increased with population density and with decreasing asexual clutch sizes. The  
20 frequency of sexually reproducing females additionally increased as population growth rates  
21 decreased. Consistent with population dynamic models showing that the opportunity cost of sexual  
22 reproduction (foregoing contribution to current population growth) diminishes as populations  
23 approach carrying capacity, we found that investment in sexual reproduction was highest when  
24 asexual population growth was low or negative. Our results support the idea that the timing of sex is  
25 linked with periods when the relative cost of sex is reduced due to low potential asexual growth at  
26 high population densities. Thus, a combination of ecological and demographic factors select on the  
27 optimal timing of sexual reproduction, allowing *D. magna* to balance the necessity of sex against its  
28 costs.

29

30 **Keywords:** Timing of sex, *Daphnia*, population density, cost of sex

31 Many treatments of the evolution of sex consider sexual and asexual forms as discrete lineages  
32 (reviewed in [1,2]). In nature, however, competition between these types is often more subtle.  
33 Organisms that use sex facultatively potentially gain the ‘best of both worlds’, as they avoid paying  
34 costs of sexual reproduction much of the time, while maintaining access to the benefits of genetic  
35 recombination [3-5]. This flexibility brings about a suite of life history consequences: the ability to  
36 reproduce either sexually or asexually, depending on current conditions, means that the frequency  
37 and timing of the sexual life cycle is an evolvable trait [6].

38 The timing of sex in facultative sexual organisms is critical to fitness for several reasons stemming  
39 from the differing demographic consequences of sexual vs. asexual reproduction. Asexual  
40 reproduction is usually the more efficient strategy in terms of converting resources into offspring, as  
41 it avoids the ‘twofold cost’ of male production [7,8]. A genotype’s asexual and sexual success are  
42 not independent, and strongly traded off against each other, because a female can only do one at a  
43 time. Switching from asex to sex too early also entails potentially large opportunity costs: foregoing  
44 the opportunity to contribute to asexual generations (i.e. current population growth) can, in an  
45 exponentially growing population, drastically reduce the representation of a clonal genotype in the  
46 mating pool, and hence in the sexually produced offspring generation. This opportunity cost (birth  
47 rate disadvantage) of sexual reproduction may be particularly pronounced when offspring gained  
48 through sex are not equivalent to offspring produced asexually, as often observed in nature. For  
49 example, in facultative sexual organisms sexual offspring are often dormant or dispersing life stages  
50 (e.g. Cladocera: [9]; rotifers [10,11]; aphids [12]) that do not contribute to current local population  
51 growth.

52 In populations that undergo periods of inhospitable conditions, an association between sexual  
53 reproduction and dormancy results in potentially complex selection on the timing of sex. Sexually  
54 produced, diapausing offspring are vital for the persistence of a lineage across favourable  
55 (‘growing’) seasons, and must be produced before the intervening periods when the habitat is  
56 unsuitable. In such a system, the measure of fitness that is expected to be maximized is the total  
57 count of sexually produced dormant stages at the end of the favourable season [13,14]. In contrast,  
58 during the growing season, selection in the short term favours asexual reproduction due to its  
59 efficiency [15]. If individuals can precisely predict the duration of each favourable season, we  
60 expect a simple switch from asexual to sexual reproduction towards the end of the growth season  
61 (Gerber, Booksmythe & Kokko, unpublished) [16,17]. However, in unpredictable environments  
62 individuals might hedge their bets with regards to reproductive mode, while in more predictable  
63 environments the use of environmental cues could allow this plasticity [18]. Indeed, in various  
64 facultatively sexual species, changes in temperature [12], population density or crowding [19,20],

65 food quality [21,22], photoperiod [22] and predation [23] contribute to the induction of sexual  
66 reproduction. All of these cues potentially inform females about seasonal changes and/or  
67 deteriorating conditions, under which sex may be advantageous [24]. The reproductive response to  
68 such cues can be complicated by the need to produce both males and sexually reproductive females  
69 so that they coincide at sexual maturity during the period optimal for the production of sexual  
70 offspring [25,26].

71 The cost of sex is not a fixed parameter, but may depend itself on current conditions. Investment in  
72 each reproductive mode is expected to be influenced by the relative costs of sexual and asexual  
73 reproduction. For example, mate-finding costs may be higher at low densities, when encounter rates  
74 between individuals are low [27,28]. However, this cost may be diminished if individuals can  
75 flexibly switch to asexuality should a mate not be found. Similarly, the cost of sex may depend on  
76 the current scope for asexual population growth [29-31]. Assuming that adults are better able than  
77 offspring to survive at high densities, when a population approaches carrying capacity the  
78 recruitment rate declines.

79 A handful of population dynamic models highlight the consequences of these dynamics for the  
80 demographic advantage of asexual lineages over sexuals [32-34]. They show that asexuality cannot  
81 always realize its demographic advantage: if high densities prevent immediate population growth,  
82 the opportunity cost of sex diminishes for populations nearing carrying capacity. Although this is  
83 not always sufficient to modify the cost if sex and asex occur simultaneously [8], the prediction for  
84 facultative sexuals with dormant sexual eggs is clear: they should switch to sex as resources become  
85 limiting and the opportunity cost of sexual reproduction is reduced. This important prediction has,  
86 to date, been largely overlooked by empirical studies of the costs and benefits of sex.

87 In facultative sexual *Daphnia* (Cladocera: Daphniidae) we expect strong selection on the timing of  
88 sex. *Daphnia* sex appears costly relative to asexual reproduction in the short term due to its  
89 demographic effects. The largest asexual clutches recorded for *D. magna* contain ~110 eggs [35],  
90 whereas sexual clutches contain at most two eggs. This clutch size difference represents a  
91 potentially extreme opportunity cost of reproducing sexually, which necessarily entails foregoing an  
92 asexual reproductive bout. Non-equivalence of sexually and asexually produced offspring holds for  
93 this system: asexually produced eggs develop immediately in the maternal brood chamber into free-  
94 swimming plankton, whereas fertilized sexual eggs must undergo a period of dormancy, encased in  
95 a hardy capsule known as an ephippium [9]. Additionally, sexual reproduction requires the  
96 (asexual) production of males, which reduces the asexual growth rate of a lineage. However, in  
97 terms of resource allocation, the extent to which investment in sex trades off with other life history

98 traits, including asexual investment, is not clear. While production times for sexual and asexual  
99 clutches are equal, the large number of eggs in an asexual clutch could translate into higher resource  
100 requirements compared to the two eggs per sexual clutch. Alternatively, melanisation of the  
101 ephippium and provisioning for dormancy might require additional resources when producing a  
102 sexual clutch.

103 Ultimately, only sexual, dormant eggs are able to withstand harsh conditions, including freezing  
104 and desiccation, so sexual reproduction is vital for the long-term persistence of a lineage over  
105 inhospitable periods. At the start of each growing season, when environmental conditions become  
106 suitable, dormant eggs hatch into females that found the planktonic population anew. Male  
107 production and the female switch to sexual reproduction may occur in response to different cues, or  
108 with different sensitivity to the same cues: in *D. magna*, production of males and of sexual clutches  
109 responded differently to manipulations of photoperiod [36], and male production has been observed  
110 to occur more stochastically throughout the growing season compared to ephippia production [25].  
111 The relative roles of environment and genotype in determining the likelihood of male and ephippia  
112 production also vary: for example, in *Daphnia pulex* inhabiting temporary ponds over a short  
113 growing season, substantial male production occurred very early while population densities were  
114 still low, and was also not linked to other environmental factors such as pond temperature [37].

115 Most studies on the timing of sex and male production in *Daphnia* have focused on cues that allow  
116 dormant eggs to be produced before environmental deterioration or the end of the season, and have  
117 been conducted under laboratory conditions (e.g. [22,26,36,38,39], but see [37]). We aim to add the  
118 costs and consequences of sex itself to this picture, and focus on population density as a variable  
119 connecting the ecological and demographic influences on the timing of sex. We highlight the  
120 hypothesis of (i) demographically varying costs of sex [32-34] as an important alternative to the  
121 prevailing emphasis on sex as a response to deteriorating conditions. This prevailing view sees sex  
122 either (ii) providing a direct escape route (e.g. dormancy [26,36]) or (iii) generating diversified  
123 offspring through recombination, to explain why particularly stressful conditions induce sex [40].

124 Of these three options, we focus on the first two (the demographic cost hypothesis, and the habitat  
125 deterioration hypothesis). The third hypothesis appears unlikely to explain the precise scheduling of  
126 sex in the current context. While high density (and its correlates, e.g. increased resource limitation  
127 or disease risk) may constitute a stressful environment, it is difficult to envisage a benefit of  
128 producing diverse offspring genotypes in response to this transient stress. Offspring hatch in  
129 subsequent seasons under benign density conditions; the range of densities a lineage may later  
130 encounter is independent of the density when the lineage-founding ephippia were produced.

131 Returning to the two focal hypotheses, previous work has shown that crowding promotes sex  
132 induction and reduces asexual fecundity in laboratory populations of *Daphnia* [41]. Observing these  
133 patterns in natural populations would support the habitat deterioration hypothesis, with support  
134 strengthening if populations do not persist after reaching high density. The demographic cost  
135 hypothesis, in contrast, predicts that density directly modifies the relative costs of sex and asexual  
136 reproduction through its relationship with the population's capacity for growth [32-34]. In this case,  
137 we would expect sex induction to be related to population growth rates in addition to density.

138 We used an intensive longitudinal sampling regime to document population dynamics and the  
139 occurrence of sexual reproduction over the main part of the growing season in natural populations  
140 of cyclically parthenogenetic *Daphnia magna*. We investigated the interacting effects of population  
141 density, asexual reproductive investment, and growth rates on the frequency of sexually  
142 reproducing individuals. Additionally, in the laboratory we estimated resource allocation trade-offs  
143 between the production of ephippia and asexual fecundity over the lifespan of individual females, to  
144 clarify whether investment in sex imposes costs beyond its immediate demographic disadvantage.

## 145 **Methods**

### 146 *Population sampling*

147 We sampled 11 natural *D. magna* populations every three-to-four days for 60 days (May 30 – July  
148 28, 2015). Populations inhabited separate rock pools distributed over 6 islands (FU1, HA, K, LON,  
149 N, and SMF) in the Finnish archipelago near Tvärminne Zoological Station (59.8420° N, 23.2018°  
150 E). We recorded density and demographic structure ('stage-structure') of the populations at each  
151 sampling point. To assess population density, 350 ml water samples were collected at 15  
152 haphazardly chosen locations spanning the pool area and depth. These were combined in a bucket  
153 and stirred to distribute individuals evenly, and a 350-ml subsample was taken as the final density  
154 sample. The remaining animals were returned to the rock pool. After collecting the density sample a  
155 small hand net was swept through the pond to take a representative population sample.

156 Live samples were brought back to the lab and analysed the same day. All *D. magna* individuals in  
157 the 350-ml density sample were counted under a dissecting microscope and converted to an  
158 estimate of individuals/L. The stage-structure samples were variable in size; to make larger samples  
159 manageable (< 1000 individuals) they were split using a Folsom plankton sample divider. The  
160 sample was then sieved through 0.6 mm nylon mesh to separate the smallest individuals.  
161 Individuals that remained in the sieve were counted and classified into the following categories  
162 under a dissecting microscope: females with asexual eggs or embryos in the brood pouch, females

163 with an empty brood pouch but filled ovaries, females with ephippia, adult females without eggs,  
164 embryos or filled ovaries, juvenile females (indicated by short 1<sup>st</sup> abdominal process [9]); adult  
165 males (prolonged first antenna, copulatory hook on the first thoracic leg [9, 42]) and juvenile males.  
166 After assessing stage-structure, up to ten females (where possible; median = 10, mean  $\pm$  SE = 8.72  
167  $\pm$  0.17) with asexual eggs in the brood pouch were isolated from the sample and maintained in  
168 individual 35 ml jars until they released their clutch. The number and sex of offspring was  
169 determined under a dissecting microscope. This paper's focus is the timing of investment in sexual  
170 reproduction, and does not present the data on offspring sex allocation, which is addressed in a  
171 second study using the population density and stage-structure data collected here (Booksmythe,  
172 Gerber, Ebert & Kokko, unpublished).

### 173 *Reproductive life history trade-offs*

174 We collected large population samples from five additional rock pool populations and isolated 60  
175 females (F0) carrying asexual clutches. Females were kept individually in 50 ml falcon tubes filled  
176 with artificial *Daphnia* medium (ADaM [43]) and fed daily with *Scenedesmus* algae (~5 million  
177 cells per individual per day) until they released their first clutch. We isolated four F1 daughters per  
178 F0 female and housed them in pairs in 50 ml falcon tubes until they produced their first clutch.  
179 Twenty-two of these groups of four sister F1 females (henceforth 'clones') synchronously produced  
180 enough daughters that we could isolate ten F2 females per clone, half of which were assigned to a  
181 long day length treatment (18:6 hours light:dark) and the other half to a short day length treatment  
182 (6:18 hours light:dark) on the day of their release from the maternal brood pouch. We used extreme  
183 day lengths (naturally occurring at midsummer and midwinter at the study site) to induce  
184 propensities for sex that were as different as possible between treatment groups. Over the 35-day  
185 experimental period, females experienced these photoperiod treatments under otherwise  
186 standardized conditions in climate chambers (20°C, with *Daphnia* placed ~20 cm below the  
187 fluorescent light source). Individual F2 females were fed and checked daily for the release of  
188 asexual clutches or sexually produced ephippia. When an asexual clutch was released, the date,  
189 number and sex of offspring were recorded, the offspring removed and the water changed. When an  
190 ephippium was produced, the date was recorded, the ephippium removed and the water changed.  
191 We recorded the date of any deaths. Females that did not reproduce were excluded from the  
192 analysis. We also excluded 7 females that produced an ephippium in the very first clutch, which  
193 needed twice the time to produce their first clutch compared to other females, indicating very  
194 unusual behaviour.



196 We were interested in how the frequency of sex relates to population density, population growth,  
 197 and asexual reproductive effort. We ran separate models to predict the frequencies of sexual  
 198 females and males, as they could respond differently to these predictors. Models for the frequency  
 199 of sexual females in a sample used population density from the previous sampling point ('lag  
 200 density') as a predictor, because these prior conditions (3-4 days before) coincide with the point at  
 201 which female reproductive mode would have been determined [9]. However, models for the  
 202 frequency of adult males used current density, as conditions at the previous sampling point do not  
 203 coincide with the production of these males. The appropriate lag period (the amount of time males  
 204 need to mature) is at least 10 days/3 sampling points, and a predictor variable using this lag would  
 205 have unacceptably reduced our sample size. Using current density in the analysis instead allows us  
 206 to examine whether males are produced so as to coincide with periods of high density in adulthood.  
 207 As density varied by orders of magnitude across populations, and within populations over time, we  
 208 used log-transformed density in all analyses. We calculated the intrinsic rate of per capita  
 209 population growth per time step as  $r = \frac{\ln(N_{t2}) - \ln(N_{t1})}{t2 - t1}$  [44].

210 Asexual reproductive effort was estimated from the clutch size of ~10 females, carrying asexual  
 211 eggs, per population sample. The clutch size among sampled females overestimates the mean  
 212 population asexual reproductive effort, as a small but variable proportion of mature females in each  
 213 population sample showed no current reproductive investment (with neither eggs in the brood  
 214 pouch nor filled ovaries). We weighted the mean clutch size of sampled females by the proportion  
 215 of currently reproductive females among all mature, non- sexually reproducing females to estimate  
 216 the mean asexual reproductive effort in the population. We used log-transformed clutch size and  
 217 reproductive effort in our analyses to normalize their distribution. Because asexual reproductive  
 218 effort and growth are related (collinearity) we included them separately in models with density as  
 219 the only other predictor. To determine the threshold asexual reproductive effort at which  
 220 populations switch to sexual reproduction, we created a binary dependent variable for whether a  
 221 population sample contained females investing in sexual reproduction or not. We fitted a logistic  
 222 regression of this variable over asexual reproductive effort and determined its inflection point.

223 Statistical analyses were performed in R (v. 3.2.2) [45]. We used linear mixed-effects models in the  
 224 package lme4 [46] for analyses of density, clutch size, and growth rates in the natural populations,  
 225 and of asexual clutch size and mean interval between clutches in the laboratory experiment. For  
 226 analyses of proportions of males and sexual females we used generalized linear mixed-effects  
 227 models (GLMMs) with binomial error and logit link in lme4. To account for repeated

measurements the population ID (natural populations) or family ID (laboratory experiment) was included as random factor. If binomial models were overdispersed an observation-level random factor was included [47]. Predictor variables in binomial GLMMs were standardized to aid in interpretation of parameter estimates, reported on the log odds scale; as an indication of effect sizes, we also present the odds ratio for each parameter, and marginal and conditional  $R^2$  [50] for each GLMM. Summary statistics are presented as mean  $\pm$  1 standard error (SE), unless otherwise specified.

235

## Results

### *Sex is associated with high density and low asexual reproductive effort*

The frequency of sexual females (adult females carrying sexual eggs) was larger following high population densities, and when mean reproductive effort among asexual females was low (Fig. 1, Table 1). The value for asexual reproductive effort at which the majority of populations contained females investing in sexual eggs was 11.47 offspring (Figure S1). The frequency of sexual females also increased with decreasing growth rate  $r$  (Fig. 1, Table 1). The frequency of males in the adult population was larger when density was high, and when asexual effort was low, but was not significantly related to population growth rate (Fig. 1, Table 1). Odds ratios in Table 1 show the predicted change in odds with each standard deviation increase in the predictor, for a constant (mean) value of the covariate. For example, the odds of a female carrying an ephippium were 0.035 at the intercept (i.e. for mean values of density and asexual effort). For each standard deviation increase in density, keeping asexual effort constant, these odds increased by a factor of 4.75.

The association of higher frequencies of sexual females with high population density and low growth rates can also be seen when looking at patterns across the growing season (Fig. S2). Across all populations, there were two main peaks in density during our sampling period. These peaks are followed by periods of reduced growth rate (Fig. S2) reflecting the negative relationship between population growth and population density (LMM: Slope =  $-0.053 \pm 0.014$ ,  $\chi^2 = 13.83$ ,  $p < 0.001$ ). Lag population density was also negatively related to asexual clutch size (LMM: Slope =  $-0.26 \pm 0.047$ ,  $\chi^2 = 31.02$ ,  $p < 0.001$ ) (Fig. S2).

### *Ephippia production trades off with asexual clutch size*

The number of females that reproduced in the long-day and short-day experimental treatments did not differ (83 of 110 and 88 of 110, respectively;  $z = 0.81$ ,  $p = 0.42$ ). The short-day treatment

successfully induced ephippia production, with 41 of 88 females producing at least one ephippium, compared to 1 of 83 females in the long-day treatment (GLMM: Slope =  $-4.700 \pm 1.199$ ,  $z = -3.92$ ,  $p < 0.001$ ; Fig. 2). Total asexual reproductive success (number of offspring) was higher in the long-day treatment (LMM: Slope =  $7.507 \pm 2.475$ ,  $\chi^2 = 9.20$ ,  $p = 0.002$ ; Fig. 2). Day length did not affect the total number of reproductive bouts, the latency to first reproduction, or the mean interval between clutches (all  $p > 0.05$ ). Nor did day length affect the mean asexual clutch size when calculated across all of a female's asexual clutches (LMM: Slope =  $0.178 \pm 0.420$ ,  $\chi^2 = 0.18$ ,  $p = 0.67$ ). Within the short-day treatment, we could compare individuals that reproduced only asexually with those that produced at least one sexual clutch. The latter had fewer total asexual offspring, losing on average 9 offspring compared to their clone mates that reproduced only asexually (asexual,  $54.1 \pm 1.9$ , sexual,  $45.4 \pm 3.2$ ; LMM: Slope =  $-9.167 \pm 3.856$ ,  $\chi^2 = 5.65$ ,  $p = 0.02$ ), but whether or not females reproduced sexually did not affect their mean asexual clutch size ( $-0.974 \pm 0.801$ ,  $\chi^2 = 1.48$ ,  $p = 0.22$ ). The absolute number of sexual clutches (0, 1 or 2) was not related to mean asexual clutch size ( $-0.523 \pm 0.588$ ,  $\chi^2 = 0.79$ ,  $p = 0.37$ ). However, the proportion of a female's reproductive events that were sexual was negatively related to her mean asexual clutch size (LMM: Slope =  $-5.958 \pm 2.535$ ,  $\chi^2 = 5.52$ ,  $p = 0.019$ ); if half of a female's clutches were sexual, the mean size of her asexual clutches was reduced by around 3 eggs compared to females producing only asexual clutches (Fig. 2c). Females that produced relatively many ephippia had smaller clutches when they reproduced asexually.

## Discussion

*Daphnia magna* in the rock pool habitat studied here experience a short growing season followed by completely inhospitable conditions in which only dormant, sexually produced eggs persist. Besides winter freezing, pools often experience summer droughts [49]. The resulting strong selection for the production of dormant eggs is therefore expected to shape the timing of sexual and asexual reproduction during the growing season. While approaching inhospitable conditions do influence investment in sexual reproduction [36], here we argue that in line with theoretical predictions, the reduced cost of sexual relative to asexual reproduction at high densities may plausibly favour density as a cue for the switch to sexual reproduction in *D. magna*. Our results suggest that an understanding of how population dynamics affect the costs of sex can inform predictions of when sex should occur, and highlight the importance of considering variation in the costs of sex when seeking explanations for the maintenance of sexual reproduction.

Investment in sexual reproduction in our sampled populations was strongly predicted by population density: when density was high at the previous sampling point, a greater proportion of females in the current sample carried sexual eggs. The frequency of males in the adult population also increased with density. These relationships were consistent over the sampled time period, where we observed two density peaks (Fig. S2). However, neither of these peaks in density and sexual reproduction was followed by extinction of the planktonic population, indicating that sex did not immediately precede inhospitable periods where the dormant stage would be vital. This conflicts with the habitat deterioration hypothesis, suggesting that the approach of inhospitable conditions alone cannot explain the marked increase in investment in sex at these points.

We suggest, in accordance with the demographic cost hypothesis, that the association of sexual reproduction and population density results from declining efficiency of asexual reproduction as populations approach carrying capacity — newborn offspring may then have difficulty recruiting into the current population, which reduces the returns on asexual offspring. Consequently, the relative profitability of sexual reproduction (which does not contribute to current population growth) increases with population density. Furthermore, sexually produced offspring are not affected by current conditions (e.g. competition), as they do not hatch until subsequent growing seasons. We could not measure juvenile survival, which is predicted to decrease with population density under this scenario. However, the frequency of sexual females increased with decreasing (or negative) population growth, and decreasing asexual reproductive effort. Thus, for a given population density, females increased their investment in sexual reproduction when potential growth was low or populations were declining. This supports the demographic cost hypothesis that sex in ephemeral *D. magna* populations is timed to coincide with periods when the opportunity cost of sexual reproduction is reduced [32-34] (in addition to the likelihood of sex increasing with ecological cues, such as changes in day length, ensuring that it occurs before the season end [36]). Our data on the clutches of females taken from natural populations suggest that this cost is balanced when the mean asexual reproductive effort in a population is around 11.5 eggs. Below this value, sexually reproducing females could be found in the majority of populations (Fig. S1).

Interestingly, in other facultative sexual systems showing an association between population density and sex induction (e.g. rotifers [14,20,50]), density-dependent induction of sex has been shown to contribute to regulating population density in a controlled laboratory setting [51]. Because of the production of males and dormant stages, which require resource investment and do not contribute to current population growth, increasing rates of sex can feed back negatively on growth rates [16,17]. In rotifers the density threshold for sex induction is low, and sex is directly related to population density, rather than indirectly through resource depletion [20], suggesting that the induction of sex

325 influences the growth potential of the population [50]. This effect on population growth has also  
326 been demonstrated in laboratory *Daphnia* populations [52]. Such negative feedback is also possible  
327 in our dataset, but is much more difficult to detect in natural populations due to the many other  
328 uncontrolled variables (e.g. pool volume, algal productivity) likely to affect rates of sex, density,  
329 and their relationship.

330 In contrast to the frequency of sexual females, the frequency of adult males was not related to  
331 population growth. High male frequencies coincided with periods of high density and low asexual  
332 reproductive effort, but generally male occurrence appears to be timed less precisely than ephippia  
333 production. This is perhaps not surprising when considering that males require time (~10 days at  
334 20°C) to mature, which reduces the likelihood that cues available when males are produced will  
335 reliably predict population dynamics at their maturity. Furthermore, as male lifespan is substantially  
336 longer than the moult cycle over which a female bears an ephippium, male frequency increases  
337 cumulatively over time while ephippia frequency reflects much more closely the current conditions.  
338 Previous studies of *Daphnia* species in temporary habitats have found similar patterns of male  
339 appearance in a population preceding the first production of ephippia [37,53].

340 High population density leads to increased investment in both males and ephippia in laboratory  
341 populations of *Daphnia* [19,54-56] and to smaller asexual clutch sizes [41]. A negative relationship  
342 between density and asexual reproduction was also apparent in our dataset. This could reflect  
343 increased competition at high densities, resulting in reduced reproductive condition that restricts  
344 female fecundity. However, we observed increased sexual reproduction at high densities, when  
345 resources are limited. This finding is difficult to reconcile with the suggestion that the sexual  
346 ephippia have a high resource cost [57]. If resources limit the production of large asexual clutches  
347 (as shown in many experiments, e.g. [40,58,59]), these conditions should also constrain production  
348 of costly ephippia. Our laboratory results on reproductive trade-offs suggest that producing a sexual  
349 clutch is costly: individual females producing a greater proportion of sexual clutches over their  
350 lifespan produced, on average, smaller asexual clutches. The cost imposed on asexual reproductive  
351 potential by a sexual event is thus greater than the loss of one asexual clutch. However, quantifying  
352 the absolute cost of producing a sexual clutch requires experiments manipulating asexual clutch  
353 sizes by altering resource availability.

354 The major cost of sex in our experiment appeared to be the immediate trade-off arising from the  
355 inability to produce a sexual and asexual clutch simultaneously: females that produced more  
356 ephippia had a lower total number of asexual offspring. If a female producing a sexual clutch has  
357 fewer opportunities and/or resources left available for asexual reproduction, there are clear

consequences for the competitiveness of clonal lineages with different propensities for sexual reproduction in terms of their numerical representation in the population. *D. magna* clones vary in their propensity to produce males and, independently, ephippia in response to environmental cues [26,36]. Sexually produced, dormant offspring are the measure of long-term fitness in *Daphnia* and many facultative sexual organisms, but total sexual output depends both on sexual and asexual fecundity. The timing of sexual reproduction is thus expected to optimise investment in the two reproductive modes.

## Conclusions

In wild populations of facultative sexual *D. magna*, females invest in sexual reproduction following high population densities and when the population growth rate and asexual reproductive effort are low, conditions that reduce the relative cost of sexual reproduction. We provide empirical support for the idea that a facultative sexual population will show increased rates of sex as it approaches carrying capacity and the cost of sex declines. Combining our new finding with previous results we suggest that three underlying rules determine the induction of sexual reproduction in *D. magna* on a large biogeographic scale: First, ephemeral, seasonal populations that frequently experience inhospitable periods should generally invest more in sexual reproduction compared to populations in permanent, less seasonal habitats [32]. Second, we have found that within a season, sex induction co-occurs with conditions that are theoretically predicted to reduce its costs relative to asexual reproduction [32-34]. This is the case at high population densities when asexual clutch size is small and the cost of foregoing asexual reproduction is low. Third, previous studies have shown that this pattern can be modified by the timing and predictability of onset of inhospitable conditions, such that investment in sexual reproduction increases towards the anticipated end of the growing season [36]. We conclude that timing of sex in cyclical parthenogens is not only shaped by the approach of inhospitable conditions, but appears to respond to effects of density and population growth on the relative costs of sexual and asexual reproduction.

## Competing interests

We have no competing interests.

## Data accessibility

All data are archived in the DRYAD digital repository (DOI provided on manuscript acceptance)

## Authors' contributions

389 NG, IB, DE and HK conceived the study; NG, IB and DE designed the study; NG and IB carried  
390 out data collection, analysed the data, and drafted the manuscript; all authors revised the manuscript  
391 and gave final approval for publication.

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## 399 **References**

- 400 1. Lively CM. 2010 A review of Red Queen models for the persistence of obligate sexual  
401 reproduction. *J. Hered.* **101**, S13-S20. (doi:10.1093/jhered/esq010)
- 402 2. Hartfield M, Keightley PD. 2012 Current hypotheses for the evolution of sex and recombination.  
403 *Int. Zool.* **7**, 192-209. (doi:10.1111/j.1749-4877.2012.00284.x)
- 404 3. Green RF, Noakes DLG. 1995 Is a little bit of sex as good as a lot? *J. Theor. Biol.* **174**, 87-96.  
405 (doi:10.1006/jtbi.1995.0081)
- 406 4. Hurst LD, Peck JR. 1996 Recent advances in understanding of the evolution and maintenance of  
407 sex. *Trends Ecol. Evol.* **11**, 46-52. (doi:10.1016/0169-5347(96)81041-X)
- 408 5. Dacks J, Roger AJ. 1999 The first sexual lineage and the relevance of facultative sex. *J. Mol.*  
409 *Evol.* **48**, 779-783. (doi:10.1007/PL00013156)
- 410 6. Stelzer C-P. 2016 Extremely short diapause in rotifers and its fitness consequences.  
411 *Hydrobiologia* online ahead of issue (doi:10.1007/s10750-016-2937-x)
- 412 7. Maynard Smith J. 1978 *The Evolution of Sex*. Cambridge, UK: Cambridge University Press.

- 413 8. Lehtonen J, Jennions MD, Kokko H. 2012 The many costs of sex. *Trends Ecol. Evol.* **27**, 172-  
414 178. (doi:10.1016/j.tree.2011.09.016)
- 415 9. Ebert D. 2005 *Ecology, Epidemiology, and Evolution of Parasitism in Daphnia*. Bethesda, MA:  
416 National Library of Medicine (US), National Center for Biotechnology Information.
- 417 10. Schröder T. 2005 Diapause in monogonont rotifers. In: Herzig A, Gulati RD, Jersabek CD, May  
418 L (Eds.) *Rotifera X: Rotifer Research: Trends, New Tools and Recent Advances, Proceedings of the*  
419 *Xth International Rotifer Symposium*. pp. 291-306. Dordrecht, NL: Springer.
- 420 11. Stelzer C-P, Lehtonen J. 2016 Diapause and maintenance of facultative sexual reproductive  
421 strategies. *Phil. Trans. R. Soc. B* **371**, 20150536. (doi:10.1098/rstb.2015.0536)
- 422 12. Simon J-C, Rispe C, Sunnucks P. 2002 Ecology and evolution of sex in aphids. *Trends Ecol.*  
423 *Evol.* **17**, 34-39. (doi:10.1016/S0169-5347(01)02331-X)
- 424 13. Taylor BE, Gabriel W. 1993 Optimal adult growth of *Daphnia* in a seasonal environment.  
425 *Funct. Ecol.* **7**, 513-521. (doi:10.2307/2390126)
- 426 14. Serra M, Aparici E, Carmona M. 2008 When to be sexual: sex allocation theory and population  
427 density-dependent growth. *J. Evol. Biol.* **12**, 263-271. (doi:10.1093/plankt/fbn077)
- 428 15. Carmona MJ, Dimas-Flores N, Garcia-Roger EM, Serra M. 2009 Selection of low investment in  
429 sex in a cyclically parthenogenetic rotifer. *J. Evol. Biol.* **22**, 1975-1983. (doi:10.1111/j.1420-  
430 9101.2009.01811.x)
- 431 16. Aparici E, Carmona MJ, Serra M. 1996 Polymorphism in bisexual reproductive patterns of  
432 cyclical parthenogens: a simulation approach using a rotifer growth model. *Ecol. Model.* **88**, 133-  
433 142.
- 434 17. Serra M, King CE. 1999. Optimal rates of bisexual reproduction in cyclical parthenogens with  
435 density-dependent growth. *J. Evol. Biol.* **12**, 263-271. (doi:10.1046/j.1420-9101.1999.00026.x)



- 436 18. Halkett F, Harrington R, Hullé M, Kindlmann P, Menu F, Rispe C, Plantegenest M. 2004  
437 Dynamics of production of sexual forms in aphids: theoretical and experimental evidence for  
438 adaptive “coin-flipping” plasticity. *Am. Nat.* **163**, E112-E125. (doi:10.1086/383618)
- 439 19. Larsson P. 1991 Intraspecific variability in response to stimuli for male and ephippia formation  
440 in *Daphnia pulex*. *Hydrobiologia* **225**, 281-290. (doi:10.1007/BF00028406)
- 441 20. Stelzer CP, Snell TW. 2003 Induction of sexual reproduction in *Brachionus plicatilis*  
442 (Monogononta, Rotifera) by a density-dependent chemical cue. *Limnol. Oceanogr.* **48**, 939-943.
- 443 21. Koch U, von Elert E, Straile D. 2009 Food quality triggers the reproductive mode in the cyclical  
444 parthenogen *Daphnia* (Cladocera). *Oecologia* **159**, 317-324. (doi:10.1007/s00442-008-1216-6)
- 445 22. Zhang L, Baer KN. 2000 The influence of feeding, photoperiod and selected solvents on the  
446 reproductive strategies of the water flea, *Daphnia magna*. *Environ. Pollut.* **110**, 425-430.  
447 (doi:10.1016/S0269-7491(99)00324-3)
- 448 23. Hairston Jr. NG, Olds EJ. 1984 Population differences in the timing of diapause: adaptation in a  
449 spatially heterogeneous environment. *Oecologia* **61**, 42-48. (doi:10.1007/BF00378705)
- 450 24. Walsh MR. 2013 The link between environmental variation and evolutionary shifts in dormancy  
451 in zooplankton. *Integr. Comp. Biol.* **53**, 713-722. (doi:10.1093/icb/ict035)
- 452 25. Galimov Y, Walser B, Haag CR. 2011 Frequency and inheritance of non-male producing clones  
453 in *Daphnia magna*: evolution towards sex specialization in a cyclical parthenogen? *J. Evol. Biol.*  
454 **24**, 1572-1583.
- 455 26. Roulin AC, Mariadassou M, Hall MD, Walser J-C, Haag CR, Ebert D. 2015 High genetic  
456 variation in resting-stage production in a metapopulation: Is there evidence for local adaptation?  
457 *Evolution* **69**, 2747-2756. (doi:10.1111/evo.12770)

- 458 27. Snell TW, Garman BL. 1986 Encounter probabilities between male and female rotifers. *J. Exp.*  
459 *Mar. Biol. Ecol.* **97**, 221-230. (doi:10.1016/0022-0981(86)90243-1)
- 460 28. Sprenger D, Lange R, Anthes N. 2011 Population density and group size effects on reproductive  
461 behaviour in a simultaneous hermaphrodite. *BMC Evol. Biol.* **11**, 107. (doi:10.1186/1471-2148-11-  
462 107)
- 463 29. Williams GC. 1975 *Sex and evolution*. Princeton, NJ: Princeton University Press.
- 464 30. Bell G. 1982 *The Masterpiece of Nature*. Berkeley, CA: University of California Press.
- 465 31. Burt A. 2000 Perspective: Sex, recombination and the efficacy of selection – was Weismann  
466 right? *Evolution* **54**, 337-351. (doi:10.1111/j.0014-3820.2000.tb00038.x)
- 467 32. Doncaster CP, Pound GE, Cox SJ. 2000. The ecological cost of sex. *Nature* **404**, 281-285.
- 468 33. Lively CM. 2010. Parasite virulence, host life history, and the costs and benefits of sex. *Ecology*  
469 **91**, 3-6.
- 470 34. Lively CM. 2011. The cost of males in non-equilibrium populations. *Evol. Ecol. Res.* **13**, 105-  
471 111.
- 472 35. Hebert PDN. 1978. The population biology of *Daphnia* (Crustacea, Daphnidae). *Biol. Rev.* **53**,  
473 387-426.
- 474 36. Roulin AC, Routtu J, Hall MD, Janicke T, Colson I, Haag CR, Ebert D. 2013 Local adaptation  
475 of sex induction in a facultative sexual crustacean: insights from QTL mapping and natural  
476 populations of *Daphnia magna*. *Mol. Ecol.* **22**, 3567-3579. (doi:10.1111/mec.12308)
- 477 37. Innes DJ. 1997. Sexual reproduction of *Daphnia pulex* in a temporary habitat. *Oecologia* **111**,  
478 53-60.

- 479 38. Spaak P, Boersma M. 2001 The influence of fish kairomones on the induction and vertical  
480 distribution of sexual individuals of the *Daphnia galeata* species complex. *Hydrobiologia* **442**, 185-  
481 193. (doi:10.1023/A:1017578221814)
- 482 39. Gyllström M, Hanson L-A. 2004 Dormancy in freshwater zooplankton: Induction, termination  
483 and the importance of benthic-pelagic coupling. *Aquat. Sci.* **66**, 274-295. (doi:10.1007/s00027-004-  
484 0712-y)
- 485 40. Hadany, L. & Otto, S.P. 2007. The evolution of condition-dependent sex in the face of high  
486 costs. *Genetics* **176**:1713-1727.
- 487 41. Fitzsimmons JM, Innes DJ. 2006 Inter-genotype variation in reproductive response to crowding  
488 among *Daphnia pulex*. *Hydrobiologia* **568**, 187-205. (doi: 10.1007/s10750-006-0104-5)
- 489 42. Dodson SI, Frey DG. 2001 Cladocera and other Branchiopoda. In: Thorp A, Covich AP (Eds.)  
490 *Ecology and Classification of North American Freshwater Invertebrates*, 2<sup>nd</sup> ed. pp. 849-913. San  
491 Diego, CA: Academic Press.
- 492 43. Klüttgen B, Dülmer U, Engels M, Ratte HT. 1994 ADaM, an artificial freshwater for the culture  
493 of zooplankton. *Wat. Res.* **28**, 743-746. (doi:10.1016/0043-1354(94)90157-0)
- 494 44. Begon M, Harper JL, Townsend CR. 1990. *Ecology* (2<sup>nd</sup> Ed.) Blackwell Scientific, Cambridge  
495 MA, pp 150-152.
- 496 45. R Core Team. 2016 R: A language and environment for statistical computing. Vienna, Austria:  
497 R Foundation for Statistical Computing. URL: <https://www.R-project.org/>
- 498 46. Bates D, Maechler M, Bolker B, Walker S. 2015 Fitting linear mixed-effects models using  
499 lme4. *J. Stat. Softw.* **67**, 1-48.
- 500 47. Harrison XA. 2014 Using observation-level random effects to model overdispersion in count  
501 data in ecology and evolution. *PeerJ* **2**, e616. (doi:10.7717/peerj.616)

- 502 48. Nakagawa S, Schielzeth H. 2013. A general and simple method for obtaining  $R^2$  from  
503 generalized linear mixed models. *Methods Ecol. Evol.* **4**, 133-142.
- 504 49. Altermatt F, Ebert D. 2008 The influence of pool volume and summer desiccation on the  
505 production of the resting and dispersal stage in a *Daphnia* metapopulation. *Oecologia* **157**, 441-452.  
506 (doi:10.1007/s00442-008-1080-4)
- 507 50. Stelzer C-P. 2012 Population regulation in sexual and asexual rotifers: an eco-evolutionary  
508 feedback to population size?. *Funct. Ecol.* **26**, 180–188. (doi:10.1111/j.1365-2435.2011.01918.x)
- 509 51. Schröder T, Gilbert JJ. 2004 Transgenerational plasticity for sexual reproduction and diapause  
510 in the life cycle of monogonont rotifers: intracloal, intraspecific and interspecific variation in the  
511 response to crowding. *Funct. Ecol.* **18**, 458-466. (doi:10.1111/j.0269-8463.2004.00854.x)
- 512 52. McCauley E, Nisbet RM, Murdoch WW, de Roos AM, Gurney WSC. 1999. Large-amplitude  
513 cycles of *Daphnia* and its algal prey in enriched environments. *Nature* **402**, 653-656.  
514 (doi:10.1038/45223)
- 515 53. Galimov Y, Walser B, Haag CR. 2011. Frequency and inheritance of non-male producing  
516 clones in *Daphnia magna*: evolution towards sex specialization in a cyclical parthenogen? *J. Evol.*  
517 *Biol.* **24**, 1572-1583. (doi: 10.1111/j.1420-9101.2011.02288.x)
- 518 54. Kleiven OT, Larsson P, Hobæk A. 1992. Sexual reproduction in *Daphnia magna* requires three  
519 stimuli. *Oikos* **65**, 197-206.
- 520 55. Berg LM, Palsson S, Lascoux, M. 2001 Fitness and sexual response to population density in  
521 *Daphnia pulex*. *Freshwater Biol.* **46**, 667-677. (doi:10.1046/j.1365-2427.2001.00704.x)
- 522 56. Olmstead AW, LeBlanc GA. 2001 Temporal and quantitative cycling of the Cladoceran  
523 *Daphnia magna* by a juvenile hormone analog. *J. Exp. Zool.* **290**, 148-155.
- 524 57. Lynch M. 1983 Ecological genetics of *Daphnia pulex*. *Evolution* **37**, 358-374.
- 525 58. Lynch M. 1989 The life history consequences of resource depression in *Daphnia*  
526 *pulex*. *Ecology* **70**, 246-256. (doi:10.2307/1938430)

527 59. Guisande C, Gliwicz ZM. 1992 Egg size and clutch size in two *Daphnia* species grown at  
528 different food levels. *J. Plankton Res.* **14**, 997-1007.

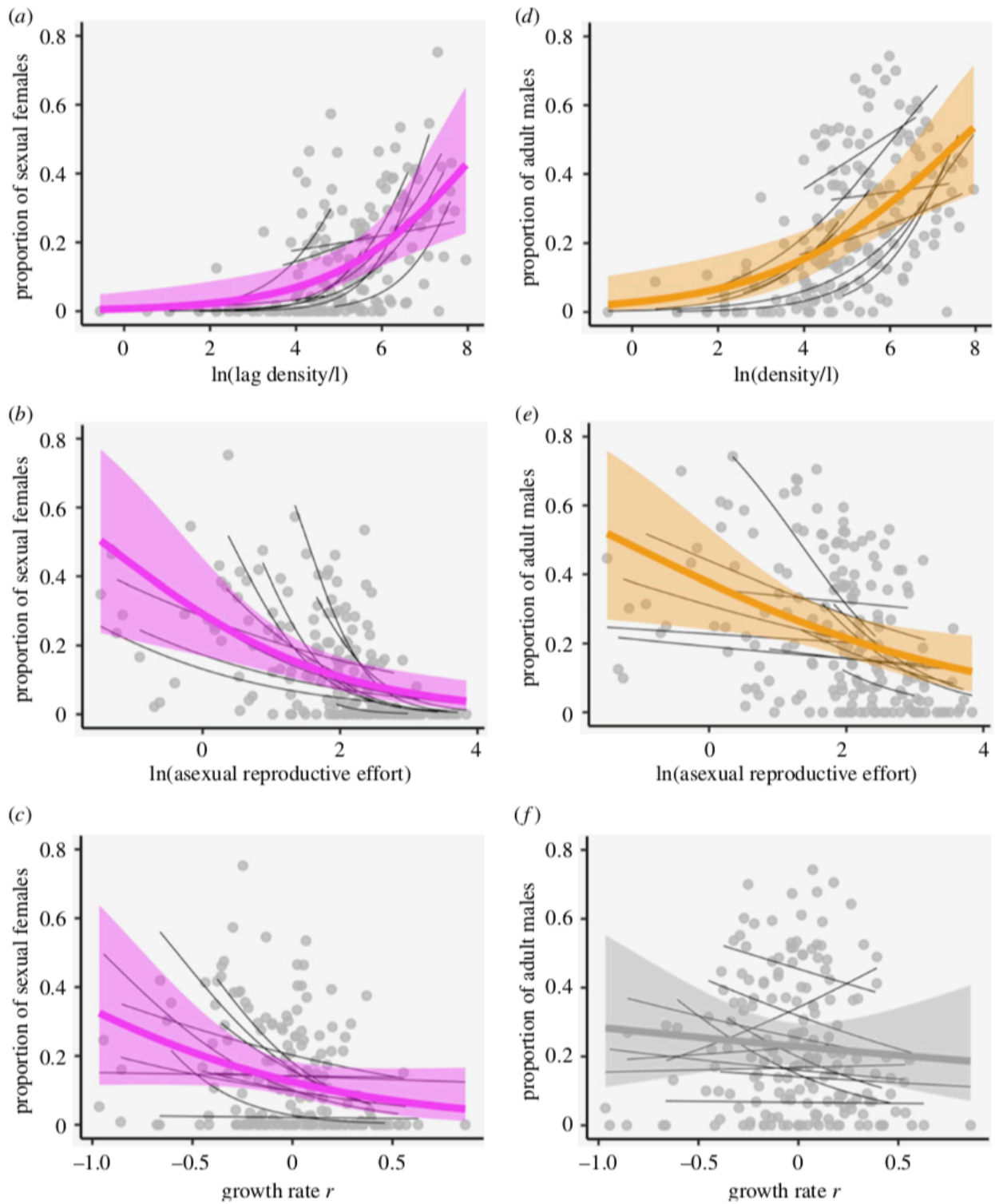
529

530 **Figure 1.** The relationship of the proportion of sexual females (a-c) and males (d-f) with lag (a) or  
 531 current (d) population density (*Daphnia*/L), asexual reproductive effort (b, e), and growth rate (c, f).  
 532 Bold lines show the global logistic regression with 95% confidence intervals, with significant  
 533 relationships in colour and non-significant in grey. Thin black lines show regressions for each  
 534 population, and light grey points show raw data.

535 **Figure 2.** The effect of day length treatment (short-day, 6:18 hours light:dark; long-day, 18:6 hours  
 536 light:dark) on (a) the mean proportion of a female's clutches that were sexual and (b) the mean size  
 537 of asexual clutches; and (c) the relationship between the proportion of a female's clutches that were  
 538 sexual and her mean asexual clutch size, for the short-day treatment only. Asterisks indicate  
 539 significance (n.s.,  $p > 0.05$ ; \*,  $p < 0.05$ ; \*\*\*,  $p < 0.001$ ).

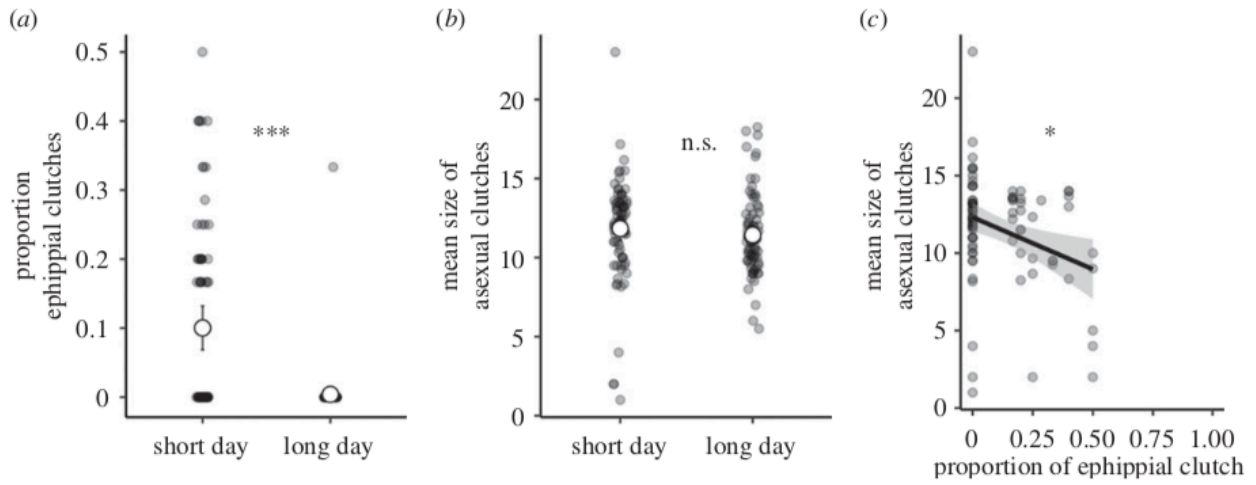
540 **Table 1.** Effects of population density, mean asexual reproductive effort (RE), and growth rate on  
 541 the proportions of sexual females and adult males in a population; estimated by binomial GLMM  
 542 with logit link. Parameter estimates are presented as the log odds ratio ( $\beta$ ) and its standard error  
 543 (SE); we additionally present the odds ratio (OR) as a measure of effect size.  $R^2_{\text{GLMM(m)}}$ , marginal  $R^2$   
 544 (variance explained by fixed effects);  $R^2_{\text{GLMM(c)}}$ , conditional  $R^2$  (variance explained by fixed + random  
 545 effects) [50].

546



547

548 **Figure 1.** The relationship of the proportion of sexual females (a-c) and males (d-f) with lag (a) or  
 549 current (d) population density (*Daphnia*/L), asexual reproductive effort (b, e), and growth rate (c, f).  
 550 Bold lines show the global logistic regression with 95% confidence intervals, with significant  
 551 relationships in colour and non-significant in grey. Thin black lines show regressions for each  
 552 population, and light grey points show raw data.



**Figure 2.** The effect of day length treatment (short-day, 6:18 hours light:dark; long-day, 18:6 hours light:dark) on (a) the mean proportion of a female's clutches that were sexual and (b) the mean size of asexual clutches; and (c) the relationship between the proportion of a female's clutches that were sexual and her mean asexual clutch size, for the short-day treatment only. Asterisks indicate significance (n.s.,  $p > 0.05$ ; \*,  $p < 0.05$ ; \*\*\*,  $p < 0.001$ ).

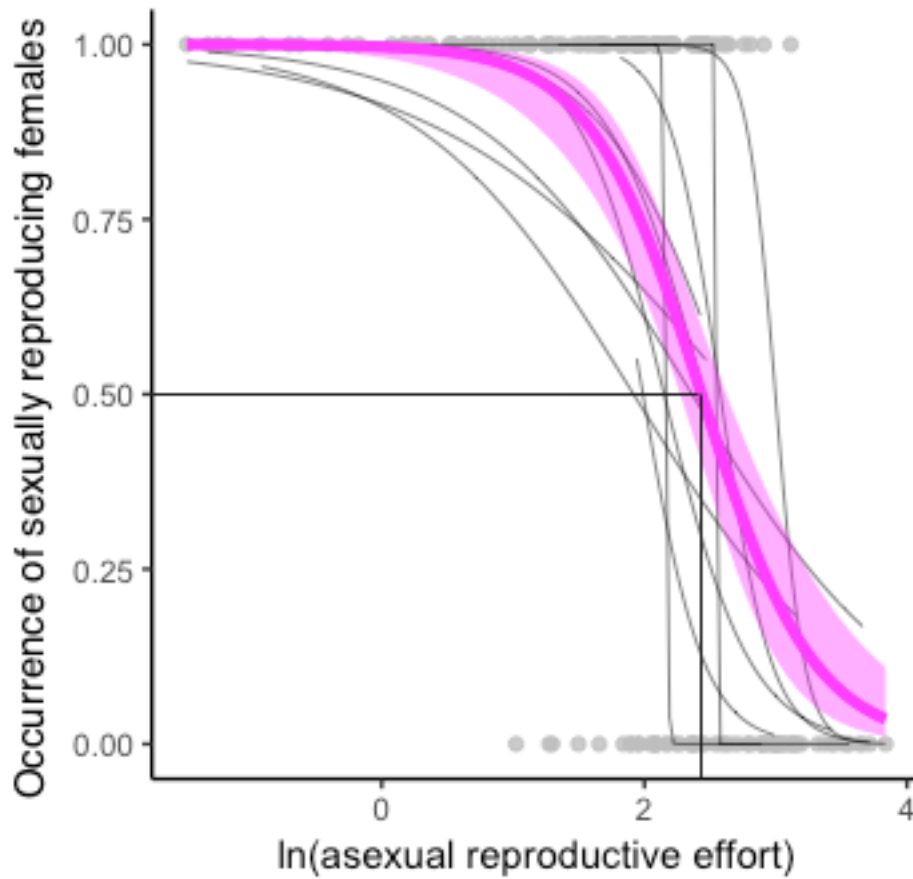


562 **Table 1.** Effects of population density, mean asexual reproductive effort (RE), and growth rate on the proportions of sexual females and adult males in a population; estimated by  
563 binomial GLMM with logit link. Parameter estimates are presented as the log odds ratio ( $\beta$ ) and its standard error (SE); we additionally present the odds ratio (OR) as a measure of  
564 effect size.  $R^2_{GLMM(m)}$ , marginal  $R^2$  (variance explained by fixed effects);  $R^2_{GLMM(c)}$ , conditional  $R^2$  (variance explained by fixed + random effects) [50].

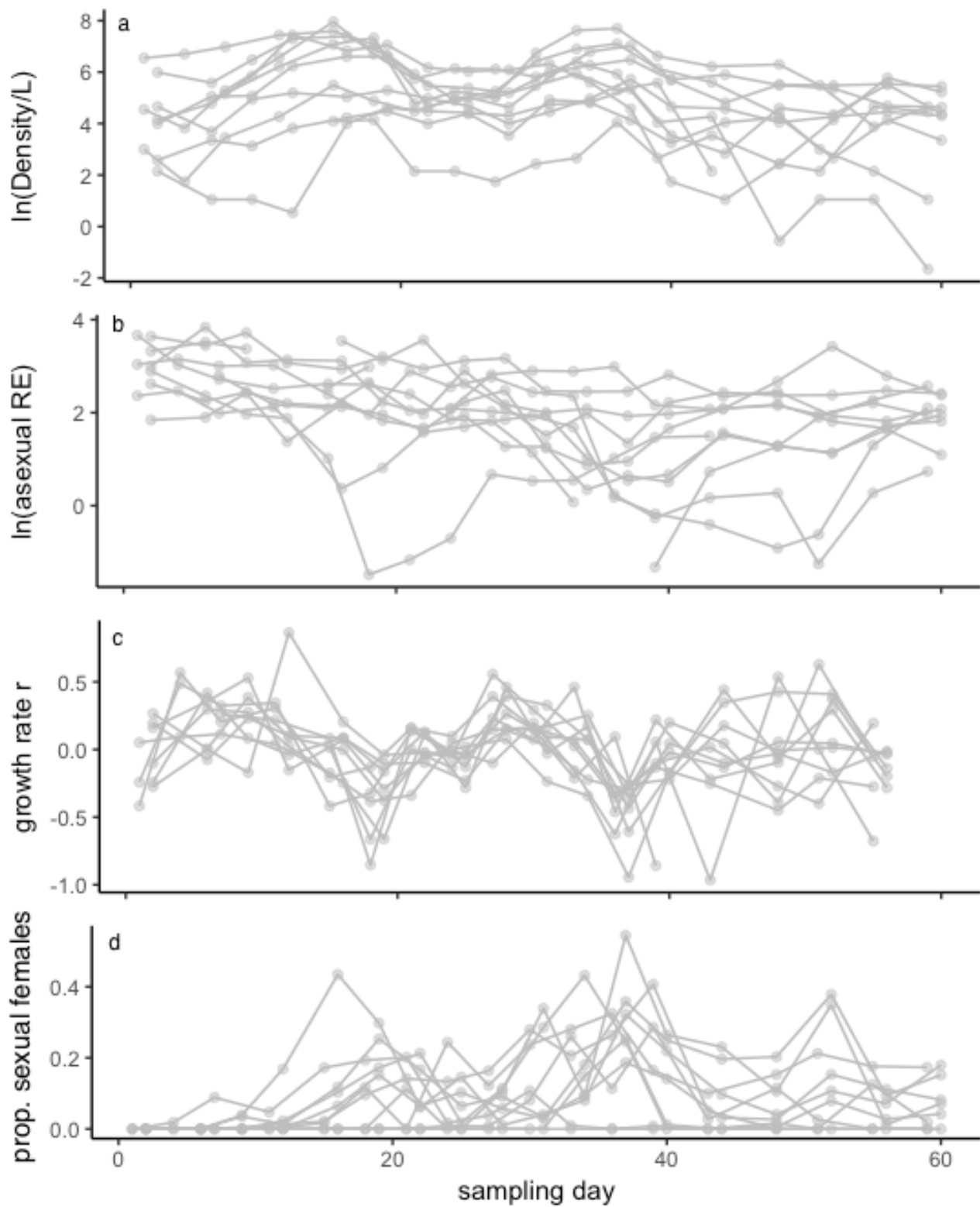
565

**Model: Density and asexual reproductive effort**

Proportion sexual females (N = 174)						Proportion adult males (N = 185)					
Fixed effects:	$\beta$	SE	z	p	OR	Fixed effects:	$\beta$	SE	z	p	OR
(Intercept)	-3.344	0.333	-10.04	< 0.001	0.035	(Intercept)	-1.878	0.172	-10.91	< 0.001	0.153
ln(lag density)	1.558	0.221	7.04	< 0.001	4.751	ln(density)	0.976	0.129	7.56	< 0.001	2.654
ln(mean asexual RE)	-0.914	0.155	-5.91	< 0.001	0.401	ln(mean asexual RE)	-0.470	0.108	-4.36	< 0.001	0.625
Random effects:	SD					Random effects:	SD				
Population ID	0.983					Population ID	0.466				
Observation ID	1.412					Observation ID	1.206				
$R^2$ :	$R^2_{GLMM(m)}$	$R^2_{GLMM(c)}$									
	0.426	0.514									



**Figure S1.** Occurrence of sexually reproducing females in the population with respect to asexual reproductive effort. Grey lines show the logistic regression within populations, whereas the pink line shows the logistic regression across populations. The inflection point (black line) indicates the threshold mean asexual reproductive effort ( $e^{2.44}=11.47$ ) at which the majority of populations contain females that carry a sexual clutch. Light grey dots show the raw data.



**Figure S2.** Temporal dynamics (day 1 = May 30, 2015) of (a) population density (*Daphnia*/L), (b) asexual reproductive effort, (c) population growth rate, and (d) the proportion of sexual females in the population. Each line shows one of the 11 rock pool populations.